# 2010 St. Croix National Scenic Riverway Zebra Mussel Monitoring and Support of Federally Listed Endangered Mussel Species



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#### **Introduction:**

The St. Croix National Scenic Riverway was the first unit of the National Park System included in the Wild and Scenic Rivers Act of 1968. The Riverway is considered a nationally significant resource for its richness and abundance of freshwater mussels (~40 species, the greatest in the Upper Mississippi watershed) and is recognized for its outstanding recreational and biological assets. The diversity of unionids within the Riverway is well documented and many threats to that diversity have been identified. This faunal group could be severely impacted by a zebra mussel (*Dreissena polymorpha*) infestation and from other invasive species. Freshwater mollusks are a keystone faunal group of freshwater systems and their potential loss is unacceptable.

In order to understand the invasion of zebra mussels into the St. Croix, measurements of density have been taken since 2004 within the known infestation zone (the last 21 miles of river). Anecdotal evidence from the Upper Mississippi River suggests zebra mussel colonization predominates on native mussel beds, especially when substrates are less favorable for recruitment (e.g., sand, silt, etc.). Therefore, sample locations were chosen based on native mussel bed survey work previously conducted by the Minnesota Department of Natural Resources (MN DNR). Six locations were identified from Stillwater, MN, to Prescott, WI, reflecting the range of habitats and hydrology found in the infestation zone. In addition, the endangered Higgin's eye pearly mussel Essential Habitat Areas of Hudson and Prescott were included. Thirty 1/8-meter quadrate samples were collected by divers at each of the locations. These samples were processed on site, frozen and examined under magnification. Data collected continues to aid managers who are creating policy based on the spread and intensity of the invasion.

In addition to understanding relative abundance of zebra mussels in Lake St. Croix, other St. Croix Basin activities for 2010 related to zebra mussels and work with endangered native mussels follows.

Qualitative zebra mussel sampling was performed in 2010 using two methods. First, throughout the watershed, including 14 locations on the main stem of the St. Croix and Namekagon and several area lakes, plate samplers with glass slides were placed in the water to encourage initial settlement of any floating veligers in the system. A second, more active sampling method involves timed SCUBA searches in areas of the river with higher probability of zebra mussel colonization, but where none, to date, have been found. These dives occurred from approximately river mile 63 to 33. During 2010, these activities were supported by the U.S. Fish and Wildlife Service (Fisheries Resources Office, Onalaska, WI), the Minnesota Department of Natural Resources and Macalester College.

Associated veliger collection continued this year as part of a broader determination of reproduction of zebra mussels throughout the Upper Mississippi River System. This monitoring is critical as a compliment to other projects in the basin in determining effects of the infestation.

Finally, the St. Croix Riverway Scuba Dive Team participated in several activities related to the propagation and recovery of the winged mapleleaf mussel (federally listed as endangered).

These activities included propagation (fish cages and lab infestations), developing mussel caches and gravid female recovery, placing and checking juvenile rearing cages, and relocation habitat assessment. We also performed mapping of the downstream distribution of the species by conducting timed dive searches.

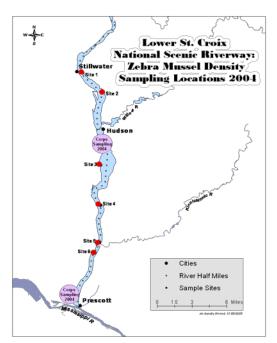
#### **Lake St. Croix Zebra Mussel Densities** (USACOE SOW Task 1)

Quantitative samples were collected at eight locations (established native mussel beds) to determine zebra mussel densities at various places within the last 21 miles of the river (Fig. 1). The locations included the Essential Habitat Areas designated for the Higgins eye mussel and representative of each of the pools and narrows of Lake St. Croix. From the confluence with Mississippi River, upstream:

- 1) Prescott Higgins eye Essential Habitat Area (EHA) (River Mile Right 0.2),
- 2) St. Croix Bluffs (RM R5)
- 3) Kinnickinnic Narrows (RM L6)
- 4) Black Bass Bar (RM L10)
- 5) Lake St. Croix Beach (RM R14)
- 6) Hudson Higgins' eye Essential Habitat Area (RM R17)
- 7) South Highline Beach (RM L21)
- 8) Stillwater Mussel Relocation Site (RM L23).

  These same eight sites were sampled in 2005-10 by NPS/FWS. In 2004, the EHAs were sampled by the USACE and WI DNR.

Figure 1. Map of eight long term zebra mussel monitoring sites established in 2004.



At each site, 30 1/8<sup>th</sup> meter-squared quadrates were sampled by haphazardly tossing the metal squares from the boat. A test dive was performed to mark suitable habitat and native mussel

densities. Within the location perimeter, the boat was moved to ensure full coverage of the native mussel bed. The dive boat was positioned in as close proximity as possible to previously sampled sub-sites locations via GPS. After all samples from the location were collected, an appropriate shore site was determined and a crew of 4-6 staff and volunteers processed each sample through 3mm screen mesh using a low pressure pump of river water. This technique (though potentially labor intensive) allowed for completion of time consuming flushing of sand, mud and small organic matter on site, without tedious transport. On site processing reduces off-site storage and contamination, aids in sample preservation, and improves efficiency.

In the laboratory, sample material was thawed and removed from the one gallon zip-locked baggies and spread onto a sorting tray. Larger debris was checked under handheld magnification and zebra mussels (if any) removed by hand or forceps. Next, the remaining material was rewashed in a 250 µm sieve then spread evenly throughout a dissecting scope and analyzed. If abundance is high, sub-samples have been used to obtain density estimates in previous years. This year's material used for density estimates was fully counted unless animals were >1mm, however. Zebra mussels were not otherwise measured for length in the 2010. Starting this year through 2012, a USGS predator/competition study began to assess growth patterns in the Lower St. Croix River from Stillwater to Prescott.

Only zebra mussels that were alive or considered living during collection were counted. All zebra mussels were placed into 4 groups: zebra mussels found attached to living native mussels were categorized for infestation rates (Fig. 2) and included for density, live zebra mussels found elsewhere in the sample quadrate were added for total density, live native mussels (identified to species) can used to calculate bed densities (Fig. 3).

2010 % of Native Mussels Infested with Zebra Mussels Prescott St. Croix Buffs 25% Kinni Narrows 50% Black Bass Bar Lake St. Croix Beach 50% Hudson 43% Hi Line Beach 20% Stillwater 60 0 20 40 80 100

Figure 2. Percent of native mussels infested with zebra mussels, by location.

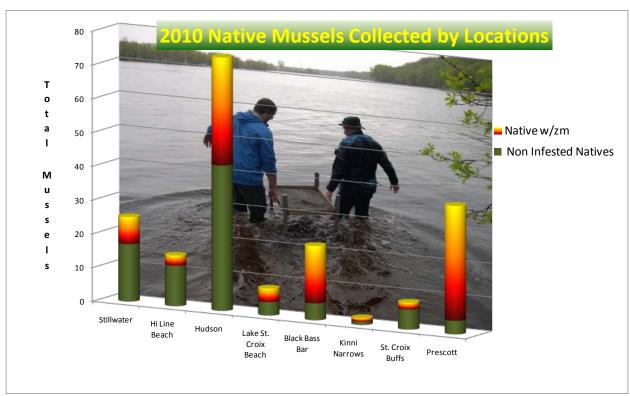


Figure 3. Live native mussels collected at eight long term monitoring sites. Total identified with and without attached zebra mussels.

Of the eight locations sampled this year, only four provided sample sizes of live native mussels large enough to consider the significance of the results as presented in Figure 2. At Hi line Beach, Lake St. Croix Beach, the Kinnickinnic Narrows, and St. Croix Bluffs, the numbers of live native mussels collected were too small to accurately judge an infestation rate. Sample size was minimally enough to assess results at Stillwater and Black Bass Bar, and these are two locations where overall zebra mussel densities increased only slightly in 2010. Here, the trend appears to be increasing infestation rates on native mussels and the changes from last year are significant. Hudson and Prescott had better sample sizes and perhaps provide stronger insight into the rates of colonizing on native mussel species. Both locations show a significant drop off in the density of live zebra mussels, however, the number of native mussels with zebra mussels attached increased from the previous year (Fig. 4).

The overall density results for 2010 reflect similar results found since data collection began in 2004. However, the comparisons for all 8 locations since that time suggest two interesting recent trends (Fig. 5). While zebra mussels remain in very low numbers in the samples from Stillwater and Bayport, there was a significant increase in densities in 2009 at Hudson, Lake St. Croix Beach and Prescott. This was not continued in 2010 (Fig. 6). Secondly while densities ballooned dramatically within the mid-reaches of the Prescott Pool in 2007 (over 12,000m² at St. Croix Buffs), there has been a significant decrease in numbers collected since then. Hydrological conditions probably drove the settlement within this pool in the past, though it is possible that ecological factors, such as predation, account for the recent lower densities.

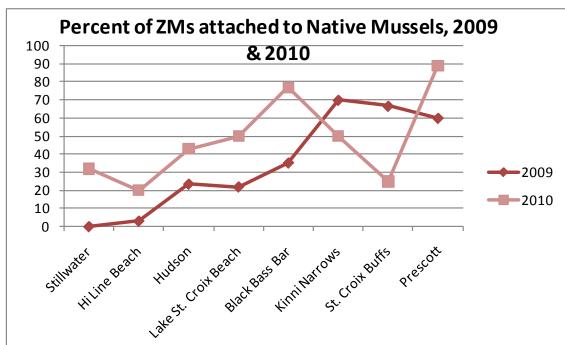
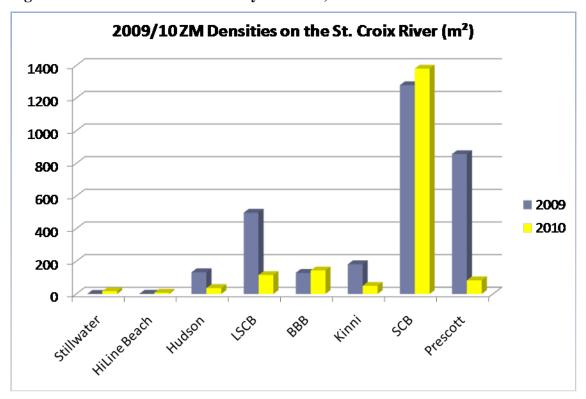


Figure 4. Native Mussels with at least one attached zebra mussel, by location, 2009 & 2010.

Figure 5. Zebra Mussel Densities by Location, 2009 and 2010



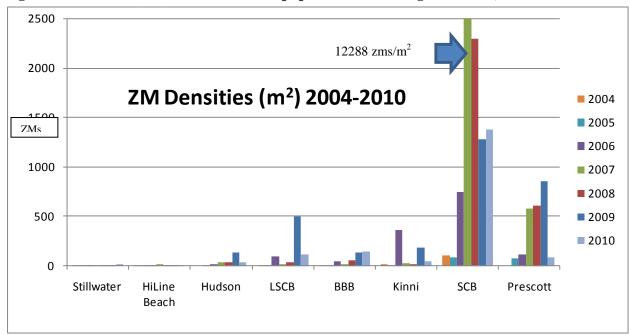


Figure 6. Densities (m<sup>2</sup>) of zebra mussel populations at 8 long term sites, 2004-2010.

As in the past additional data was collected as zebra mussel densities were determined. These included Asian clams (*Corbicula fluminea*), any species of snails and dead mussels or their shells. The results of the clam and snail enumeration can be found in the Appendix D. The Asian clam data showed an unusual spike in the number of shells collected at the St. Croix Buffs location in both 2009 and 10. The reason for this is beyond the scope of this report, but may be analyzed at a later date. The vast majority of the snails collected were of the Family Pleuroceridae.

Zebra mussel densities have fallen off from their 2007 peak in the lowest, or Prescott Pool. In the middle of this pool, at St. Croix Bluffs, densities, while lower, have leveled off. The Prescott sampling site saw a dramatic decrease in live zebra mussels, however, empty shells continue to be plentiful. This die-off appears to be the result of fish predation – most of the live native mussels were captured with multiple byssus affixed – and this phenomena is currently being explored in a related USGS Study.

# **Zebra Mussel Veliger Qualitative Monitoring** (USACOE SOW Task 2)

The FWS continues to add to the efforts of the NPS by providing PVC samplers at locations on the river and at Wisconsin lakes at high risk to the river (Table 1). These samplers are in addition to the fourteen PVC samplers with glass slides deployed by the NPS during the summer season. None of the NPS samplers (located from Stillwater to the headwaters and includes the Namekagon) had a positive detection in 2010. The Fish and Wildlife efforts include several samplers at marina on the lower St. Croix. All four of the marina located below the lift bridge in Stillwater, proved again to contain the results of reproduction, though with a decrease amount of settlement travelling upstream. None of the three marinas upstream of the lift bridge provided a

Table 1. USFWS qualitative zebra mussel veliger monitoring

FY 2010 ZM Plate Sampler Monitoring and ANS Outreach (USFWS Dave Wedan-La Crosse FWCO)						
Location	County	#Plates	# zms found	<b>Public Contacts</b>	Times slides pulled	
Afton Marina	Wash	3	Yes numerous	7		
Hudson Marina	St. Croix	3	Yes numerous	25+		
Bayport Marina	Wash	4	Yes numerous	20+		
Sunnyside Marina	Wash	2	Yes some	10+		
Stillwater Yacht Club	Wash	4	None	20+		
Wolf's Marina	Wash	4	None	20+		
Boomsite Landing	Wash	2	None	30+		
Totals	7	22	Numerous	132	N/A	
Mallalieu	St. Croix	4	None	20+	2 (neg)	
Cedar	St. Croix-Polk	3	None	10+	2 (neg)	
Big Lake	Polk	2	None	10+	2 (neg)	
Wapagassett	Polk	4	None	30+	2 (neg)	
Deer	Polk	3	None	30+	2 (neg)	
Long	Polk	3	None	10+	2 (neg)	
Balsam	Polk	6	None	30+	2 (neg)	
Half Moon	Polk	4	None	20+	2 (neg)	
Bone	Polk	4	None	25+	2 (neg)	
Big Round	Polk	2	None	5	2 (neg)	
Big Butternut	Polk	3	None	25+	2 (neg)	
Totals	11	38	0	215+	22	
Monitoring Dates; Apr 21/22, May 6/7, May 27/28, June 2/3, June 22/23, July 8/9, July22/23, Aug 4/5, Sept 7/8, Sept 22/23, Oct 4/5, Oct 26/27, Nov 1/2, Nov 10/11						

positive sampler. Several boats examined at these marinas in the fall had attached zebra mussels, but were contained on boat being stored from downstream moorings. No zebra mussels have been found by passive sampler or active scuba north of the lift bridge.

FWS staff also deployed PVC samplers at eleven Wisconsin lakes within the St. Croix watershed and draining directly into the river. These samplers were checked 14 times from April through November. No zebra mussel juveniles were detected. Also during site visits, staff engaged in outreach related to aquatic invasive species and ways to reduce their spread.

New in 2010, various lakes within the watershed and on both sides of the river were tested for veliger presence using new technology associated with the FlowCAM (Table 2). This

Table 2. Qualitative veliger sampling using plankton tows at fourteen lakes within the St. Croix Basin, July, 2010.

Veliger Samples (plankton tows)						
Processed by Clean Lakes, Inc.						
_		Clam Falls				
SC10002	16-Jul	Flowage	No veligers detected - Abundant organisms			
SC10003	15-Jul	North Center Lake	No veligers detected - Abundant organisms			
SC10005	16-Jul	Big Trade Lake	No veligers detected - Scanty organisms			
SC10010	13-Jul	Pokegama Lake	No veligers detected - Very Abundant organisms			
SC10013	14-Jul	Balsam Lake	No veligers detected - Scanty organisms			
SC10012	14-Jul	Deer Lake	No veligers detected - Scanty organisms			
SC10011	14-Jul	Bone Lake	No veligers detected - Scanty organisms			
SC10001	16-Jul	Yellow Lake	No Detect with Inconclusive Images			
SC10004	15-Jul	St. Croix Flowage	No Detect with Inconclusive Images			
SC10006	13-Jul	East Rush Lake	No Detect with Inconclusive Images			
SC10007	19-Jul	Memory Lake	Negative			
SC10008	19-Jul	Minerva Flowage	No Detect with Inconclusive Images			
SC10009	13-Jul	Cross Lake	No Detect with Inconclusive Images			
SC10014	26-Jul	Kost Dam	Negative			

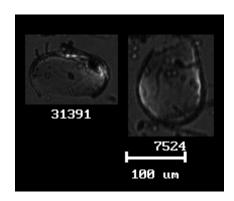
microscope speeds the processing of sampling remarkably and provides surprisingly fast results. NPS staff collected samples in July, using protocols developed by Clean Lakes, Inc. These samples were shipped overnight to company facilities and a report was generated with a couple of weeks. An example is provided below (Fig. 7). Samples were collected using a 20cm Wisconsin tow net, a 1 to 3 meter vertical pull at three locations per lake (mid and 2 littoral), integrated and preserved in ethanol. During these events, shoreline searches were conducted to detect for the presence of adult zebra mussels. No adults or shells were found along the shore.

No veligers were detected by Clean Lakes from the 14 samples provided. This strategy will be expanded during the 2011 field season. The cover page of the Clean Lakes report is provided in Appendix B. The full report is available as a pdf upon request.

# Figure 7. Descriptive analysis of veliger samples collected in July 2010 by NPS and processed by Clean Waters, Inc.

SC 10001 - Yellow Lake

This sample has been labeled as a No Detect result w/ Inconclusive Images. A positive identification picture from a Lake Mead Sample is shown above in Figure 2. The images below are from Yellow Lake and an explanation as to why we believe each image is or is not a Dressinid veliger. Some of these images are meant to show the difficulties with providing positive identification as well as some pictures commonly found in the samples.



#### Sample Information:

Client Sample ID, Date Sample Collected, Date Sample Received, Detail Sample Location, Clean Lakes Sample Number, Date Sample Analyzed, Amount Tested (mL)

Image 31391 is a similar shape to a bivalve, but is exhibiting weak birefringence lacking the Maltese Cross pattern, is translucent, and is not quite the characteristic "D" shape of a Dressinid veliger. This is not a Dressinid veliger.

Image 7524 is a similar shape to a bivalve, but is exhibiting weak birefringence lacking the Maltese Cross pattern, is translucent, and is not quite the characteristic "D" shape of a Dressinid veliger. This is not a Dressinid veliger.

# **Zebra Mussel Veliger Quantitative Monitoring** (USACOE SOW Task 3)

A multi-agency effort to collect water samples to quantify zebra mussel is conducted in July and August each summer. Again this summer, staff from NPS visited the long term monitoring locations affect the St. Croix River. Results are pending and will be contained in a separate report provided by the ACOE, ERDC office. Results from 2009 are included in Appendix C.

#### Goals for 2011

Goals for 2011 will be avaible shortly with the interagency scope of work now being developed by the USACOE, FWS, NPS and others. The above three Tasks will be included for 2011, as well as Task 4 and 5 contained in the report below (Appendix A).

#### APPENDIX A

St. Croix River Winged Mapleleaf Conservation Project – 2010 Final Report



Live winged mapleleaf collected at William O'Brien State Park, MN



Pair of young winged mapleleaf valves collected at William O'Brien State Park



Old winged mapleleaf valves collected at William O'Brien State Park



Brooding winged mapleleaf being Bagged for propagation program

Submitted to: Byron N. Karns

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#### January 2011

#### 1.0 Executive Summary

We completed four objectives to improve winged mapleleaf conservation efforts in the St. Croix River during 2010. Over the past century the winged mapleleaf has experienced reductions in range and abundance and the federal government responded by listing it as endangered in 1991. The Winged Mapleleaf Mussel Recovery Plan lists, among other activities, re-establishing winged mapleleaf populations and identifying the current range of the species as tasks that will contribute to its delisting. We undertook four projects to assist with conservation efforts: (1) aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in propagating juveniles, (2) collect brooding winged mapleleaf for use in propagating juveniles at Genoa National Fish Hatchery, (3) survey the lower St. Croix River for unknown populations of winged mapleleaf, and (4) assess survivorship of pustulous mussels marked in 2009 at two locations in the upper St. Croix River under consideration as winged mapleleaf reestablishment sites. We followed standard methods to conduct these studies. SCUBA was used to aggregate and collect winged mapleleaf at Interstate State Park that were used in the 2010 juvenile winged mapleleaf propagation program at Genoa National Fish Hatchery. SCUBA surveys of 15 locations in the lower St. Croix River revealed a previously unknown population of winged mapleleaf at William O'Brien State Park, which expanded the known range of Q. fragosa in the St. Croix River by 30%. We observed mixed results in our efforts to recapture pustulous mussels marked in 2009. We recaptured 38 marked mussels at the Nevers Dam site where one individual had died, yielding an estimated 3% annual mortality of marked mussels. In contrast, we did not find any of the marked mussels at the Sunrise River site. We believe we either mis-recorded the location of the Sunrise River mussels in 2009 or perhaps the mussels were covered with sediment. Completion of these objectives helped move forward efforts to conserve winged mapleleaf.

#### 2.0 Introduction

Winged mapleleaf (*Quadrula fragosa*), a native mussel once found throughout much of the upper Mississippi River, has become extirpated throughout most of its historic range and is in need of conservation efforts. Listed as federally endangered in 1991 (USFWS 1991) and the Winged Mapleleaf Recovery Plan lists several activities needed to delist the species including describing the species' current range, re-establishing and strengthening winged mapleleaf populations (*e.g.*, using propagated juveniles), and identifying locations where winged mapleleaf can be re-established (USFWS 1997). This project addressed these conservation needs through the following objectives: (1) aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in juvenile propagation efforts, (2) collect brooding winged mapleleaf from the St. Croix River for the production of juvenile winged mapleleaf at Genoa National Fish Hatchery, (3) survey the lower St. Croix River for unknown populations of winged mapleleaf, and (4) assess survivorship of marked pustulous mussels aggregated in 2009 at two locations in the upper St. Croix River being considered for re-establishing winged mapleleaf populations.

#### 3.0 Methods

#### 3.1 Winged mapleleaf aggregation

We worked with employees of the US Fish and Wildlife Service (USFWS), National Park Service (NPS), and Minnesota Department of Natural Resources (MN DNR) to aggregate winged mapleleaf in the St. Croix River at Interstate State Park for use in juvenile mussel propagation at Genoa National Fish Hatchery, Genoa, WI. We used SCUBA and snorkeling equipment in July 2010 to collect winged mapleleaf and relocate some of them to pre-established USFWS aggregations located just downstream of the Folsom Island wing dam. Winged mapleleaf we did not relocate were given to the USFWS for marking and placement in aggregations.

#### 3.2 Winged mapleleaf retrieval

Working with USFWS, NPS, and MN DNR employees we retrieved winged mapleleaf from aggregation sites at Interstate State Park, as well as finding new winged mapleleaf not in the aggregations, for use in juvenile mussel propagation. We assisted with winged mapleleaf collection dives weekly between August-September 2010 in the St. Croix River and also helped with transporting brooding winged mapleleaf to Genoa National Fish Hatchery.

#### 3.3 Winged mapleleaf distribution

During 2010 we surveyed sites between Otisville, MN and Prescott, WI in the St. Croix River using SCUBA in an effort to find unknown populations of winged mapleleaf. Two to four divers spent 5-60 min searching for mussels at each of 15 sites. At most sites two searches were undertaken. The first was a timed search where divers collected all living mussels and empty valves until collection bags were full (5-15 min) for use in comparing relative abundance and diversity of the mussel community among sites. The

remainder of the time spent at the site was used to search for additional mussel species (live and dead) in order to better describe the species composition of the local mussel community. During all aspects of the survey emphasis was placed on finding living or dead winged mapleleaf. Living and dead mussels collected at each site and a qualitative description of the substrate was recorded.

#### 3.4 Mussel recapture

We revisited two locations where we had marked 100 pustulous mussels during 2009 in order to recapture them or their valves and assess annual survivorship. We used SCUBA to search for marked mussels at Nevers Dam (521322E 504224N) and upstream of the Sunrise River boat launch (510175E 504944N). We spent a day searching at each of the two sites. All living and dead marked animals were counted and returned to the collection site.

#### 4.0 Results

#### 4.1 Winged mapleleaf aggregation

Working with the USFWS, NPS, and MN DNR we collected and relocated winged mapleleaf to aggregations downstream of the wing dam at Folsom Island, Interstate State Park, St. Croix River. We spent four days assisting with this effort. The USFWS, Twin Cities Field Office kept a tally of winged mapleleaf that were collected and relocated (Appendix A).

#### 4.2 Winged mapleleaf retrieval

We checked winged mapleleaf aggregations regularly during the fall of 2010 and obtained four gravid individuals that were used for juvenile propagation efforts. Agencies and organizations that planned to participate in this effort planned to aggregate winged mapleleaf between Aug 23-Oct 1. Mark Hove was scheduled to work between Aug 27-Oct 1 (and worked between Aug 27-Sept 24) but flooding occurred in the St. Croix River between Sept 24-Oct 4 and the search for brooding winged mapleleaf had to be stopped. Four brooding winged mapleleaf were collected during 2010 both in and outside of aggregation areas (Fig. 1). Mark Hove assisted with transportation of gravid animals to Genoa National Fish Hatchery. Additional details about brooding winged mapleleaf used in the 2010 St. Croix River winged mapleleaf propagation effort is available from the USFWS, Twin Cities Field Office.



Figure 1. A brooding winged mapleleaf observed outside of aggregation area downstream of Folsom Island wing dam at Interstate State Park, St. Croix River. (Photographs taken by Nick Rowse, USFWS)

#### 4.3 Winged mapleleaf distribution

We used SCUBA to survey 15 sites in the St. Croix River between July 28-Sept 16, 2010 in an effort to find unknown populations of winged mapleleaf. During this survey we observed 24 live unionid species and 24 species represented by empty valves between Otisville, MN-Prescott, WI (Fig. 2). The only place where we observed winged mapleleaf was at William O'Brien State Park where we collected one live (Fig. 3) and three dead (Fig. 4) animals. Living and dead mussels collected at each site and a qualitative habitat description was recorded (Appendix B).

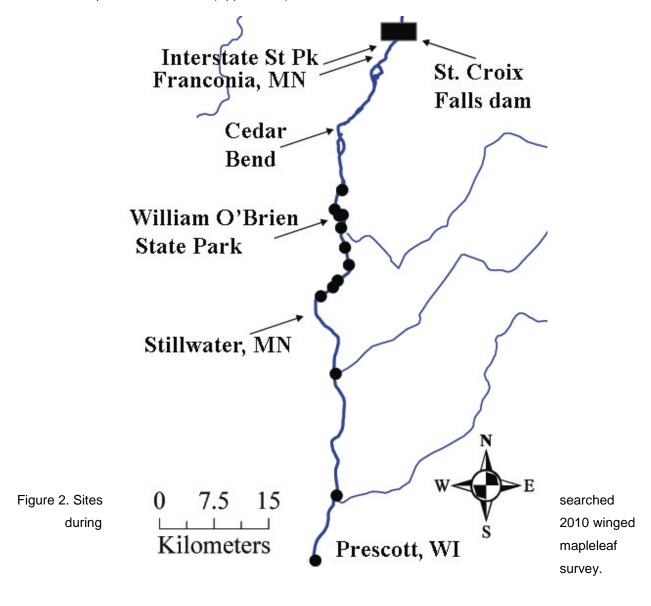




Figure 3. Live winged mapleleaf observed at William O'Brien State Park, St. Croix River.



Figure 4. Empty winged mapleleaf valves collected at William O'Brien State Park, St. Croix River: (a) external view of specimen 1, (b) internal view of specimen 1, (c) external view of specimen 2, (d) internal view of specimen 2, (e) external view of specimen 3, (f) internal view of specimen 3.

#### 4.4 Mussel recapture

During July 2010 we searched for pustulous mussels marked in 2009 at the potential winged mapleleaf re-establishment sites at Nevers Dam and Sunrise River in the upper St. Croix River. We found some marked mussels at the Nevers Dam site but none were observed upstream of the Sunrise River boat launch (Fig. 5). A total of 37 of 100 live mussels marked in 2009 and one dead marked mussel was found at the Nevers Dam site (3% annual mortality), but no live or dead marked mussels were observed at the Sunrise River site. Divers observed very few mussels at the Sunrise River site nor did they feel mussels as they searched the substrate.

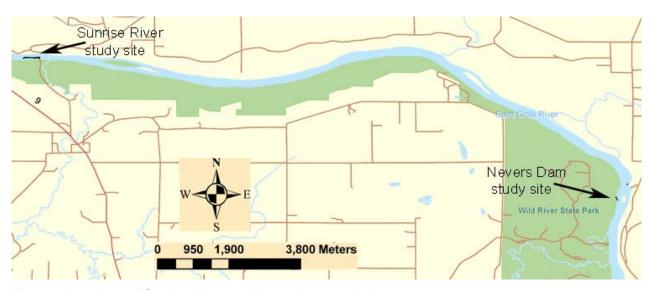


Figure 5. Locations of Sunrise River and Nevers Dam study sites.

#### 5.0 Discussion

Our efforts to find undiscovered winged mapleleaf populations in the lower St. Croix River was successful. We searched several reaches along 62 km of the lower St. Croix River that looked promising for winged mapleleaf. We sought areas similar to where we have found winged mapleleaf at Interstate State Park and Franconia, MN, *i.e.*, reaches downstream of deep pools with coarse, stable substrate. We did not find any deep pools downstream of Cedar Bend but we found that river thalwegs were good locations for finding coarse substrate and mussels. Along a gentle thalweg at William O'Brien State Park we found a previously unknown group of winged mapleleaf. This site is 9 km downstream of the furthest downstream site previously known for to hold winged mapleleaf at Cedar Bend (Heath 2002), an approximate 30% extension of the known range for the species in the St. Croix River. Although we did not observe any other winged mapleleaf populations the most promising habitats we observed occurred upstream of the High Bridge. Sites in Lake St. Croix appeared rather poor for winged mapleleaf, that is, substrate at most sites was primarily sand with only scattered areas of coarse substrate. It would be

useful to know if winged mapleleaf at Cedar Bend and William O'Brien State Park are self-sustaining or if they are maintained by animals upstream.

Efforts to aggregate and find brooding winged mapleleaf during 2010 were successful. Over the last several years we've often collected around 5 brooding winged mapleleaf for use in propagating juvenile mussels. This year we visited winged mapleleaf aggregation areas more frequently to increase the likelihood of collecting more brooding animals. The team searching for brooding winged mapleleaf this year and found four individuals but our collection efforts were compromised by a flood, which made the river inaccessible for the last quarter of the winged mapleleaf brooding period.

Recovery of pustulous mussels marked in 2009 was variable between sites at Nevers Dam and Sunrise River during 2010. At both sites we checked the location of the aggregation using GPS several times through the search, as well as verifying the location visually with markers along on the shore. Additionally, divers searched throughout and 15 m beyond the aggregation area. We recovered 38 (1 dead) of 100 marked mussels at Nevers Dam site, and none at Sunrise River site. Since we found no living or dead marked mussels at Sunrise River site we may have searched the wrong portion of the river due to an error in recording the aggregation location during 2009. Alternatively, the mussels may have moved away from the aggregation site but it would be unusual for the majority of the mussels to move (Cope et al., 2003). There was guite a bit of shifting sand at the Sunrise River site, which may have buried the mussels at the site although divers also felt for mussels in the sand during search efforts. During 2009 we placed marked mussels in high quality habitat at both Sunrise River and Nevers Dam sites. It is difficult to understand why we did not find the marked mussels at the Sunrise River site. Our observations reinforce the merits of making multiple GPS readings with high quality GPS reader. Additionally, mussel aggregations could be marked with an underwater marker (e.g., 20 m rope staked between two dog screws oriented parallel with river current) although this may draw the attention of the public to the aggregation.

#### 6.0 Acknowledgments

We thank the St. Paul District Army Corps of Engineers for project funding and the National Park Service for administering the funds.

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#### APPENDIX B

For
St. Croix National Scenic Riverway
Submitted to
St. Croix National Scenic Riverway
c/o Byron Karns
401 Hamilton Way
St Croix Falls, WI 54024
Submitted by
1770 West State Street #125
Boise, ID 83702

**INTRODUCTION:** Clean Lakes, Inc. recently completed analysis of fourteen (14) water samples for the St. Croix National Scenic Riverway to determine the presence or absence of Dressinid veligers. Clean Lakes, Inc. utilized FlowCAM ® technology with a 300um x Field of Vision flow cell, 4 x objective, and cross polarized light source optimized for maximum veliger birefringence. During visual examination of the sample picture files, we did detect birefregent organisms, though no positive Dressinid veliger identification was possible. We are including several images that are representative of organisms with similar features to Dressinid veligers with an explanation of features that disqualify each from positive identification.

BACKGROUND INFORMATION: Positive identification of Dressinid veligers (Zebra and Quagga mussels) using standard or FlowCAM® microscopy has certain limitations which require further testing using PCR technology for final confirmation of species. The main limiting factor for positive identification using microscopic based technologies is the wide variety of common bivalves that may exist in a waterbody that can have similar characteristics to the Dressinid family of bivalves. Of particular concern are Ostracods and Corbicula bivalves, both of which share similar traits with zebra and quagga mussels, including birefringence. However, there are physical characteristics that can be used to differentiate between the species, with shell shape being the primary characteristic. A good graphic showing the difference between Dressinid mussel veligers and Ostracods is shown in Figure 1 with descriptions of differentiating characteristics. Figure 1 also depicts other challenges to providing 100% positive identification, including mussels on edge and other particles such as sand that can produce birefringence.

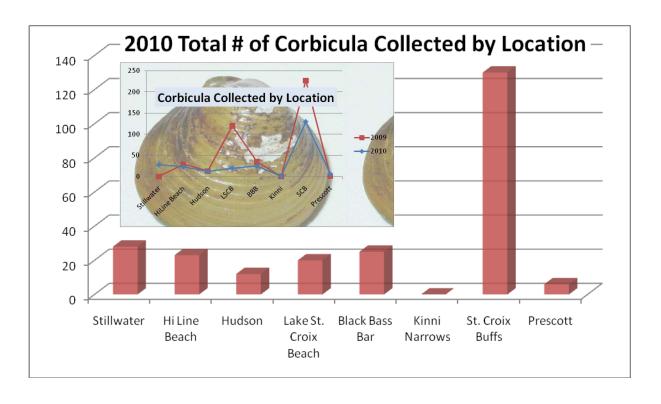
FULL REPORT CAN BE OBTAINED AS A PDF FILE WITH GRAPHICS

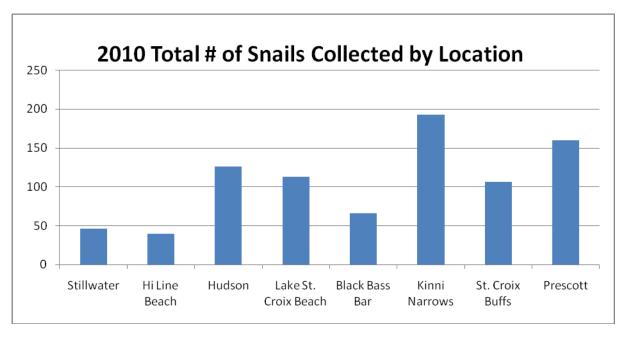
# APPENDIX C

# **2009 Tributary Veliger Collection**

		Live Veligers (#/L)		
	Tributaries	July	August	
Minnesota	Snake River	0	0.13	
Upstream of	Sunrise River	0.02	0	
winged mapleleaf	St. Croix River -St. Croix Falls	0.02	0.02	
Minnesota/Wisconsin	St. Croix R Prescott	34.1	4.76	
	St. Croix R Hudson	25.02	10.91	
	St. Croix River - Stillwater	0	0.02	
Wisconsin	Chippewa R Hwy 35	0	0	
	Black R Hwy 35	-	-	
	Wisconsin R.	0	0.09	
lowa	Wapsi R mouth	0	0	
	Maquoketa River	1.39	0.11	
	Iowa R mouth	0	0	
	Cedar R mouth	0	0	
Illinois	Chicago Area	-	-	
	Peoria/LaGrange	-	-	
	Alton Area	-	-	
	Rock River	0		

## APPENDIX D





# APPENDIX E

## Zebra Mussel Qualitative Sampling St. Croix National Scenic Riverway 2010



Report to Minnesota Department of Natural Resources

From National Park Service, St. Croix National Scenic Riverway

March 2011

#### **Background:**

Zebra mussels have been a threat to the St. Croix watershed since the early 1990. In 1992, the first mussels were discovered in the Mississippi above the confluence with the St. Croix River. The first boat discovered with attached zebra mussels was in 1994 and reproduction was pinpointed by 2000. The need for extensive monitoring within the watershed to determine the rate and extent of zebra mussel spread is extremely important. While determining control methods and mitigation is a high priority for all stakeholders, a broad list of continuing monitoring efforts is in place. This summary details the Scuba dive qualitative monitoring on the River during the summer of 2010. No zebra mussels were found upstream of the Stillwater Lift Bridge.

Several years ago, the Minnesota DNR and the National Park Service agreed on a monitoring strategy employing qualitative scuba diving to determine the range extent and relative abundance of zebra mussels in the St. Croix River. The following is a listing of those dive events occurring during the summer of 2010. This effort satisfies the requirements of agreement No. GA6590006-RM02.

#### **Results:**

8/17 Nevers Dam

15T 521529.09 m E; 5042658.67 m N

Two divers for 92minutes

8/17 Interstate SP

15T 526316.65 m E; 5026920.05 m N

Two divers for 139 minutes

8/30 St. Croix Falls Flowage

15T 521224.37 m E; 5041001.67 m N

Two divers for 30 minutes

15T 523996.85 m E; 5036320.37 m N

Two divers for 30 minutes

15T 527421.11 m E: 5030370.38 m N

Two Divers for 60 minutes

#### 8/31 St. Croix Falls Flowage

15T 527296.24 m E; 5029081.37 m N

Three Divers for 97 minutes

#### 9/3 Interstate

15T 526405.14 m E; 5026956.84 m N

Two divers for 60 minutes

15T 526254.61 m E; 5026702.17 m N

Three divers for 72 minutes

#### 9/9 William O'Brien

15T 519305.07 m E; 5006497.01 m N

Two divers for 90 minutes

#### 9/10 Interstate

15T 525314.60 m E; 5025339.92 m N

Four divers for 480 minutes

#### 9/13 Interstate

15T 526316.65 m E; 5026920.05 m N

Two divers for 120 minutes

#### 9/14 William O'Brien

15T 518889.98 m E; 5006106.95 m N

Two divers for 120 minutes

#### 9/16 Osceola

15T 522713.80 m E: 5018607.76 m N

Two divers for 22 minutes

15T 520123.91 m E; 5015959.83 m N

Two divers for 46 minutes

15T 518898.21 m E; 5011245.38 m N

Two divers for 70 minutes

#### 9/24 Interstate

15T 526405.14 m E; 5026956.84 m N

Two divers for 110 minutes

#### 9/29 Interstate

15T 526172.20 m E; 5026454.02 m N

Two divers for 150 minutes

9/30 Stillwater

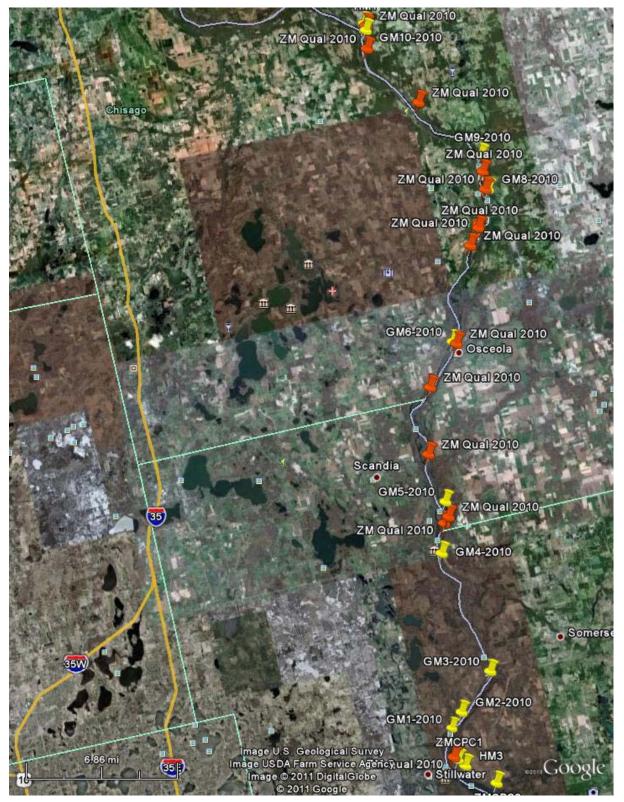
15T 515729.63 m E; 4989368.85 m N

Two divers for 180 minutes

For the 2010 qualitative zebra mussel monitoring on the St. Croix River, the St. Croix Dive Team covered 15 locations during 19 visits. The approximately 42 miles of river search, represented 12 days and a similar number of river habitat. In the water search time represented 31.6 diver hours. Six adult zebra mussels were discovered attached to riprap or native mussels at the Stillwater site, but no zebra mussels were found upstream of the Stillwater lift bridge. In addition, semi-quantitative sampling (plankton tows and FlowCam ®assessments) of water just north of Stillwater, at St. Croix Falls, and above the Snake River confluence, along with samples taken in the Snake, Sunrise and various other lakes and impoundments directly connected to the St. Croix, did not detect veligers in 2010.

Figure 1. General Location of the Twelve Qualitative Zebra Mussel Sampling Locations, 2010

Figure 1. General Location of the Twelve Qualitative Zebra Mussel Sampling Locations, 2010



## APPENDIX F

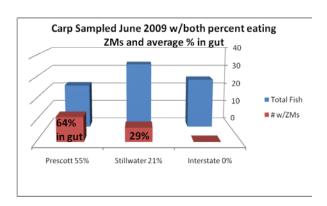
# Analysis of Common Carp and Drum Stomach Contents

Table 1. Gut contents of carp and drum collected from three lower river locations in June and August of 2009.

2009 Fish Stomach Analysis: Percent of Specimens Consuming Zebra Mussels (ZMs) & Relative Abundance of ZMs in Gut								
	Carp							
	June				August			
	Total Fish	# w/ZMs	%	Gut %	Total Fish	# w/ZMs	%	Gut %
Interstate	25	0	0	0	28	0	0	0
Stillwater	33	7	21%	29%	28	11	39%	78%
Prescott	22	12	55%	64%	27	22	81%	89%
	Drum							
	June August							
	<b>Total Fish</b>	# w/ZMs	%	Gut %	<b>Total Fish</b>	# w/ZMs	%	Gut %
Interstate	5	0	0	0	2	0	0	0
Stillwater	*	*	*	*	9	3	33%	49%
Prescott	0	0	0	0	5	1	0	0

<sup>\*</sup>Data Pending

Figure 1. Amount of zebra mussels within the stomachs of summer sampled carp collected at three locations



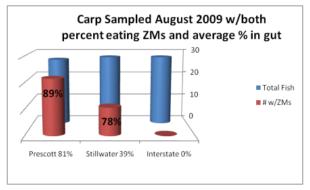
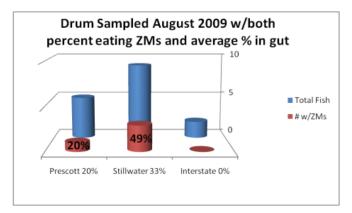


Figure 2. Zebra mussels within the guts of freshwater drum collected at three location in August 2009.



Full tables are available on request. Data includes specimens by species, location and date, with percent of zebra mussels, native mussels, detritus and other. Each fish was measured, weighed and sexed.